Journal of Forensic and Legal Medicine 23 (2014) 12–18

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

Measurement of β-tryptase in postmortem serum in cardiac deaths

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ARTICLE INFO

Article history:
Received 20 August 2013
Received in revised form
19 December 2013
Accepted 19 January 2014
Available online 25 January 2014

Keywords:
Tryptase
Mast cell
Acute coronary syndromes
Sudden death
Coronary thrombosis
Myocardial infarction

ABSTRACT

Mast cells are well known for their role in hypersensitivity reactions. However, there is increasing evidence that they might also participate in both developing and weakening atherosclerotic plaques, potentially causing plaque instability. Some clinical studies have therefore postulated the existence of relationships between blood β-tryptase levels and acute coronary syndromes. In this study, we investigated postmortem serum β-tryptase levels in a series of 90 autopsy cases with various degrees of coronary atherosclerosis that had undergone medico-legal investigations. β-tryptase concentrations in these cases were compared to levels observed in 6 fatal anaphylaxis cases following contrast material administration. Postmortem serum β-tryptase concentrations in the anaphylactic deaths ranged from 146 to 979 ng/ml. In 9 out of 90 cases of cardiac deaths, β-tryptase levels were higher than clinical reference values of 11.4 ng/ml and ranged from 21 to 65 ng/ml. These results indicate that increased postmortem serum β-tryptase levels can be observed, though not systematically, in cardiac deaths with varying degrees of coronary atherosclerosis disease, thereby suggesting that mast cell activation in this disease cannot be ascertained by postmortem serum β-tryptase measurements.

1. Introduction

Human mast cells are well known for their participation in acute systemic hypersensitivity reactions. In addition to this role, there is increasing evidence of their possible participation in the pathogenesis of atherosclerosis disease.[1,2]

Sporadic mast cells are normally present in the myocardium as well as in the adventitial layer of the coronary arteries. They also accumulate in the shoulder regions of atherosclerotic plaques and at the actual sites of plaque erosion or rupture. Inflammatory cells, including mast cells, tend to increase with the clinical severity of the coronary syndrome. Indeed, at the immediate plaque erosion or rupture site the number of activated mast cells was found to be up to 200-fold higher than in unaffected areas of the same coronary artery.[3–7]

Mast cells produce a number of inflammatory mediators including histamine, prostaglandins, tryptase and chymase as well as a variety of cytokines and chemokines. Neutral proteases such as tryptase and chymase are capable of triggering extracellular matrix degradation via activation of matrix metalloproteinases. Tryptase itself, and the activated matrix metalloproteinases, can degrade various components of the pericellular/extracellular matrix, thereby predisposing plaques to a weakening of the fibrous cap and subsequent erosion or rupture.[2–4]

Serum tryptase concentrations in acute coronary syndromes have been tested in some clinical reports with, at times, inconsistent results among the different research groups.[2–4,8]

In the realm of clinical and forensic pathology, only a few studies have investigated mast cell activation within atherosclerotic coronary arteries and coronary plaques in autopsy cases.[4–7,9–12]

In this study, β-tryptase concentrations in postmortem serum from femoral blood was investigated in a series of autopsy cases with various degrees of coronary atherosclerosis (minimal, severe and with acute thrombosis/myocardial infarction) that had undergone medicolegal investigations. The main goal of this study was to assess whether or not these deaths were associated with increased mast cell activity in the atherosclerotic plaque, reflected by increased postmortem serum β-tryptase levels. Furthermore, we wished to investigate whether β-tryptase concentrations in these cases were quantitatively similar to those measured in anaphylaxis-related deaths.

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1752-928X/$ – see front matter
http://dx.doi.org/10.1016/j.jflm.2014.01.009

2. Material and methods

2.1. Study design

The present study was a prospective, single centre study, performed during 2008–2013. All cases collected for the study had undergone medicolegal investigations that were performed upon request by inquiring authorities (the public prosecutor) in order to reconstruct the sequence of events leading to death. Postmortem angiography, complete conventional autopsy, histology, neuropathology, toxicology, microbiology and biochemistry were performed in all the subjects included in the study after unenhanced computed tomography scan. Conventional autopsies were performed by two forensic pathologists (at least one board-certified) within 24 h after body discovery respecting both local standards and international guidelines for medico-legal autopsies. Case inclusion criteria consisted of postmortem interval (not exceeding 48 h) and availability of femoral blood, postmortem serum from femoral blood and vitreous humor during autopsy. Biological samples from bodies with severe decompositional changes were rejected. No specimens were excluded due to insufficient sample volume.

2.2. Study population

A total of 96 subjects (67 males and 29 females), with a mean age of 53.6 years (range 16–78 years), were selected for this study. After having performed all postmortem investigations, 4 groups were identified.

The first group (Group A) consisted of 30 cases (20 males, 10 females, age range 16–42 years) including apparently healthy individuals who had suddenly died in the presence of witnesses, with minimal coronary atherosclerosis at postmortem examination. All individuals included in this group had undergone cardiopulmonary resuscitation attempts. Based on the results of all postmortem investigations, the cause of death was considered cardiac arrhythmia/cardiac arrest in the absence of macroscopic and microscopic abnormalities other than minimal coronary artery atherosclerosis.

The second group (Group B) consisted of 30 cases (20 males and 10 females, age range 51–78 years) including individuals with severe coronary artery atherosclerosis, coronary artery calcifications and severe luminal narrowing as well as myocardial fibrosis at postmortem examination, without acute coronary thrombosis or myocardial infarction. Based on the results of all postmortem investigations, the cause of death was determined to be cardiac arrhythmia/cardiac arrest in the presence of severe coronary artery atherosclerosis and myocardial fibrosis.

The third group (Group C) consisted of 30 cases (22 males, 8 females, range 46–69 years) including individuals with coronary artery atherosclerosis, acute coronary thrombosis and acute myocardial infarction at postmortem examination. Based on the results of all postmortem investigations, the cause of death was determined to be myocardial infarction in the presence of acute coronary thrombosis.

The fourth group (Group D) consisted of 6 cases (5 males, 1 female, range 53–79 years) of fatal hypersensitivity reaction following contrast material exposure that underwent medicolegal investigations. Based on the results of all postmortem investigations, the cause of death was determined to be anaphylactic shock.

2.3. Heart dissection

Hearts were sectioned before or after fixation in 10% neutral buffered formalin. The major epicardial coronary arteries were either serially sectioned at approximately 5 mm intervals or longitudinally sectioned intact on the heart. In the latter, segments of interest were removed and decalcified if needed before paraffin processing.

2.4. Histology

Conventional histology included hematoxylin-eosin (HE) stain of the brain, heart, coronary arteries, lung, liver and kidneys. Mast cells, degranulated mast cells and eosinophils in the coronary arteries were specifically sought out throughout Pagoda Red (PR) stain.[13] HE and PR stains were performed after tissue fixation in formaldehyde.

Paraffin sections of the left and right heart ventricles were examined for evidence of ischemia or infarction. Full thickness areas involving the left anterior, lateral free wall and left posterior ventricle as well as interventricular septum, and the right anterior, lateral free wall and right posterior ventricle were sampled. Acute infarction was defined as myocyte necrosis with or without acute inflammatory cells and/or focal granulation tissue measuring at least 1 cm^2.

Most coronary arteries with thrombi were embedded serially in paraffin and divided into segments maintaining proximal-to-distal orientation. Histological sections were prepared at three different equally spaced levels in order to best identify plaque rupture sites. Routine histology stains for heart tissues and coronary arteries included HE and PR. Immunohistochemistry was not performed.

2.5. Toxicology and biochemistry

Toxicology consisted of ethanol determination in blood and general unknown screening for common drugs and illegal substances in blood and urine by gas chromatography-mass spectrometry (GC-MS) using commercial mass spectrum libraries, high-performance liquid chromatography with diode-array detection (HPLC-DAD) and headspace-gas chromatography flame ionization detection (HS-GC-FID).

Biochemical investigations included measurement of β-tryptase, total IgE, cardiac troponin I, N-terminal pro-brain natriuretic peptide (NT-proBNP), procalcitonin and C-reactive protein in postmortem serum from femoral blood collected during autopsy as well as determination of β-hydroxybutyrate in femoral blood collected during autopsy. Biochemical investigations also consisted of vitreous sodium, chloride and glucose measurements.

All the aforementioned investigations are systematically performed in our facility.

2.6. Sample collection

Biological samples for toxicology and biochemistry were obtained as soon as possible on arrival of the bodies at the morgue (vitreous humor) and during postmortem examination (femoral blood and postmortem serum).

Undiluted vitreous humor samples (between 1 and 3 ml) were obtained by aspiration using a sterile needle and syringe. Right and left vitreous humor samples were collected through a scleral puncture at the lateral canthus, aspirated from the centre of each eye, pooled in the same syringe and mixed together. After collection, vitreous samples were immediately centrifuged at 3000 g for 15 min. The separated supernatant was collected and stored in preservative-free tubes. No specimens were excluded due to insufficient sample volume. All samples were transferred to the laboratories immediately post collection.

Femoral blood samples were collected by aspiration with a sterile needle and syringe from the femoral vein(s) after

unenhanced CT-scan and before autopsy. Blood samples were drawn after clamping the vein(s) at the proximal end and lifting the lower limb(s) for several minutes. Samples were stored in tubes containing sodium fluoride and preservative free gel serum separator tubes. These latter were centrifuged immediately post collection at 3000 g for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in preservative free tubes. Femoral blood and postmortem serum samples were transferred to the laboratories immediately after collection.

2.7. Laboratory assays

β-hydroxybutyrate concentrations were determined in femoral whole blood anticoagulated with sodium fluoride on a Cobas Mira Plus (Roche Diagnostics, Switzerland) by an enzymatic photometric method adapted in house from the technique described by Ruell and Gass.[14] Blood samples thawed overnight at 4 °C were deproteinized with perchloric acid and supernatant was used for analysis.

NT-proBNP and procalcitonin were measured in postmortem serum from femoral blood with the commercially available immunoassays on the Roche Modular E170 system (Roche Diagnostics GmbH, Mannheim, Germany).

cTnI was analyzed in postmortem serum from femoral blood with the Access® AccuTnI™ assay on Access II (Beckman Coulter, Fullerton, CA, USA).

CRP (immunoturbidimetric Tina-quant CRP) was determined using the Roche standard methods on the Roche Modular P system (Roche Diagnostics GmbH, Mannheim, Germany).

Mast cell β-tryptase was measured in postmortem serum from femoral blood with a commercial fluoroenzyme immunoassay (FEIA) method (Pharmacia & Upjohn, Freiburg, Germany) with a detection limit below 2 ng/ml. Total IgE were determined in postmortem serum from femoral blood with a commercial fluoroenzyme immunoassay (FEIA) method (Pharmacia & Upjohn, Freiburg, Germany).

Glucose, sodium and chloride were determined on vitreous humor samples by enzymatic assays on a Dimension® Xpand® Plus Integrated Chemistry System (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA).

2.8. Ethical issues

All relevant ethical issues were identified and discussed with the local Ethical Committee. All cases collected for this study underwent medicolegal autopsies as requested by the public prosecutor. Toxicology, histology and biochemistry, including β-tryptase measurements, were performed as part of the medicolegal investigations. All biological samples were anonymized prior to analysis. No further ethical approval was necessary to perform biochemical investigations in the selected cases.

2.9. Reference values

Reference value of serum β-tryptase in living subjects is 11.4 ng/ml. In forensic pathology routine, increased β-tryptase concentrations in postmortem serum over the clinical cutoff value may be a relatively common finding in allergic and nonallergic deaths.[15] Hence, based on the observations of Mayer et al.,[15] Edston et al.[16] and Fineschi et al.,[17] the value of 45 ng/ml was chosen as the reference value to discriminate anaphylactic from nonanaphylactic deaths. Blood, postmortem serum and vitreous humor reference values for the other tested parameters were the following: blood β-hydroxybutyrate 50–170 μmol/l, postmortem serum C-reactive protein 10 mg/l, postmortem serum procalcitonin 0.06 μg/l (2 μg/l for the diagnosis of sepsis), postmortem serum troponin I <0.03 μg/l or ng/ml, postmortem serum NT-proBNP <115 ng/l, postmortem serum IgE 5–50 kU/l, vitreous sodium 135–145 mmol/l or mEq/l, vitreous chloride 98–110 mmol/l or mEq/l, vitreous glucose 10 mmol/l for the diagnosis of hyperglycemia.

3. Results

Postmortem serum β-tryptase, troponin I and NT-proBNP concentrations concerning each studied group are summarized in Table 1. Non-contributory or negative results obtained from postmortem investigations performed in each case (including negative histology, toxicology or biochemistry) are not reported as they failed to provide additional diagnostic information to the interpretation of the other data.

In Group A (minimal coronary atherosclerosis without myocardial infarction), the cause of death was determined to be natural death based on the results of all postmortem investigations. Toxicology was negative in all cases included in this group. Pathologic examination of coronary arteries generally revealed some degree of atherosclerosis consisting of lipid plaques. Systemic mastocytosis was not observed. In 29 out of 30 cases, β-tryptase levels were lower than the clinical reference value of 11.4 ng/ml. In only one case, β-tryptase concentration (65 ng/ml) was higher than the clinical reference value of 11.4 ng/ml and higher than the value of 45 ng/ml proposed to discriminate anaphylactic from non-anaphylactic deaths. Total IgE were lower than the clinical reference value (5–50 kU/l) in all cases. Mild increases in postmortem serum troponin I levels (higher than 0.03 μg/l) were observed in most of the cases included in this group and were considered consistent with minimal myocyte damage due to cardiopulmonary resuscitation attempts or early decompositional changes.

In Group B (severe coronary artery atherosclerosis without acute coronary thrombosis or myocardial infarction) the cause of death was determined to be natural death based on the results of all postmortem investigations. Toxicology failed to provide the

Table 1
Postmortem serum β-tryptase, troponin I and NT-proBNP concentrations in each studied groups.

	β-Tryptase (ng/ml)	Troponin I (μg/l)	NT-ProBNP (ng/l)
Group A			
Minimum value	3	0.04	65
Maximum value	65	0.14	114
Median	7	0.08	95
Mean	9	0.08	93
Group B			
Minimum value	3	0.09	121
Maximum value	48	1.15	303
Median	8	0.46	186
Mean	9	0.55	191
Group C			
Minimum value	3	0.86	155
Maximum value	51	6.18	331
Median	7	2.38	193
Mean	12	2.78	209
Group D			
Minimum value	146	0.16	145
Maximum value	979	1.01	197
Median	176	0.52	162
Mean	318	0.56	165

Group A – Minimal coronary atherosclerosis without myocardial infarction (30 cases).
Group B – Coronary atherosclerosis and myocardial fibrosis without myocardial infarction (30 cases).
Group C – Severe coronary atherosclerosis, acute coronary thrombosis and myocardial infarction (30 cases).
Group D – Fatal anaphylaxis to contrast material (6 cases).

cause of death in all cases included in this group. Pathologic examination of heart and coronary arteries revealed various extents of myocardial fibrosis as well as fibroatheromatous plaques with variable degrees of chronic inflammatory infiltrates leading to partial or complete luminal stenosis. In 27 out of 30 cases, β-tryptase levels were lower than the clinical reference value of 11.4 ng/ml. In 3 cases, β-tryptase levels (21 ng/ml, 48 ng/ml, 26 ng/ml) were higher than the clinical reference value of 11.4 ng/ml. Total IgE were lower than the clinical reference value (5–50 kU/l) in all cases. In one case, β-tryptase concentration (48 ng/ml) was higher than the value of 45 ng/ml proposed to discriminate anaphylactic from non-anaphylactic deaths. Increases in postmortem serum troponin I and NT-proBNP values (higher than 0.03 μg/l and 115 ng/l, respectively) were systematically observed in all cases included in this group, suggesting myocyte damage due to cardiopulmonary resuscitation attempts, ischemic myocyte necrosis as well as preexisting or terminal myocardial insufficiency. Systemic mastocytosis was not observed. Mast cells, degranulated mast cells and eosinophils within coronary plaques were observed on occasion, especially in those cases with increased β-tryptase levels (Figs. 1 and 2).

In Group C (severe coronary artery atherosclerosis, acute coronary thrombosis and acute myocardial infarction) the cause of death was determined to be natural death based on the results of all postmortem investigations. Toxicology failed to provide the cause of death in all cases included in this group. Pathologic examination of heart and coronary arteries revealed various extents of myocardial necrosis and myocardial fibrosis as well as acute luminal thrombi and ruptured plaques with variable degrees of mixed inflammatory infiltrates leading to partial or complete luminal stenosis (left circumflex 11 cases, left anterior descending 11 cases, right coronary 8 cases). In 25 out of 30 cases, β-tryptase levels were lower than the clinical reference value of 11.4 ng/ml. In 5 cases, β-tryptase levels (39 ng/ml, 30 ng/ml, 51 ng/ml, 46 ng/ml and 48 ng/ml) were higher than the clinical reference value of 11.4 ng/ml. Total IgE were lower than the clinical reference value (5–50 kU/l) in all cases. In 3 cases, β-tryptase concentrations (51 ng/ml, 46 ng/ml and 48 ng/ml) were higher than the value of 45 ng/ml proposed to discriminate anaphylactic from nonanaphylactic deaths. Increases in postmortem serum troponin I and NT-proBNP values (higher than 0.03 μg/l and 115 ng/l, respectively) were systematically observed in all cases included in this group, indicating ischemia-induced myocyte necrosis as well as preexisting or terminal myocardial insufficiency. Systemic mastocytosis was not observed. Mast cells, degranulated mast cells and eosinophils within coronary plaques were observed on occasion, especially in those cases with increased β-tryptase levels.

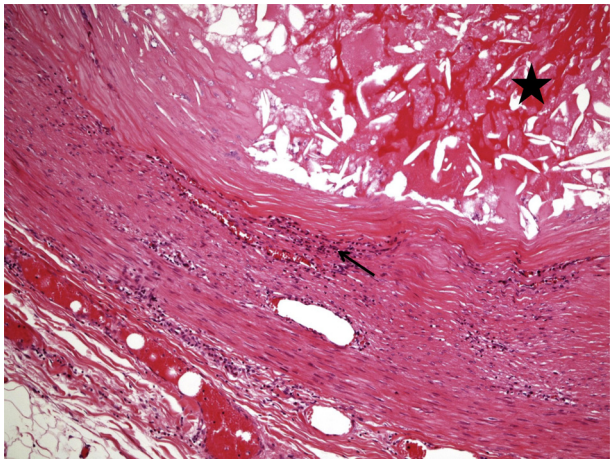

Fig. 1. Section of left anterior descending coronary artery. Chronic inflammatory infiltrate (black arrow), deposit of fibrinoid material and cholesterol crystals (black star). Hematoxylin-eosin (HE) stain. Original magnification 10×.

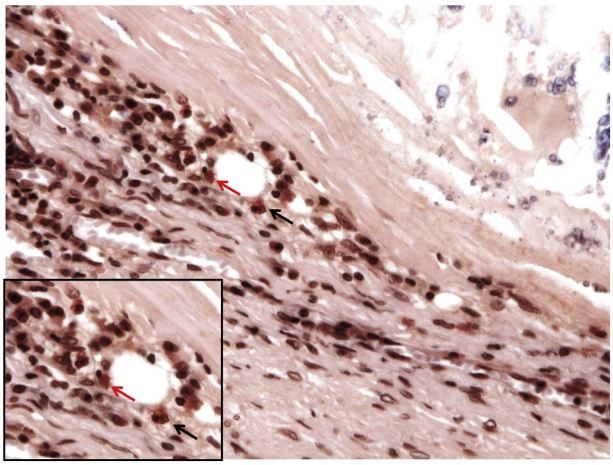

Fig. 2. Section of left anterior descending coronary artery. Chronic inflammatory infiltrate, deposit of fibrinoid material and cholesterol crystals. Pagoda Red (PR) stain. Original magnification 20×. In the box, eosinophils (black arrow), mast cells and degranulated mast cells (red arrow). Pagoda Red (PR) stain. Original magnification 40×.

In Group D (anaphylaxis-related death following contrast material exposure) the cause of death was determined to be anaphylactic shock based on the results of all postmortem investigations. Toxicology failed to provide the cause of death in all cases included in this group. A significant increase in eosinophils, mast cells and degranulated mast cells in the spleen, the latter mainly located in spleen sinuses, was detected in all cases included in this group. Mast cells, degranulated mast cells and eosinophils within coronary arteries were not observed. Postmortem serum β-tryptase levels ranged from 146 to 979 ng/ml. Biochemical investigations revealed increases in troponin I and NT-proBNP levels (higher than 0.03 μg/l and 115 ng/l, respectively) in all cases, consistent with myocardial cell damage possibly due to myocardial ischemia during prolonged hypotension, cardiopulmonary resuscitation attempts or terminal heart failure preceding electromechanical dissociation.

As expected, postmortem serum β-tryptase levels were significantly higher in anaphylaxis-related deaths than in cardiac deaths (Fig. 3). Postmortem serum β-tryptase levels higher than 45 ng/ml were identified in 5 cardiac deaths (1 individual with minimal atherosclerosis, 1 individual with severe atherosclerosis and 3 individuals with severe atherosclerosis with acute thrombosis). No statistically significant differences pertaining to β-tryptase levels were observed among cardiac deaths with minimal atherosclerosis (Group A), severe atherosclerosis (Group B) and severe atherosclerosis with acute thrombosis (Group C).

4. Discussion

Mast cells are often considered as tissue basophils, particularly numerous in connective and submucosal tissues. In these tissues, they have been shown to play a key role in the regulation of innate and adaptive immune responses, immunity against pathogens and angiogenesis. Importantly, mast cell products play a role in regulating vascular functions. Mast cells are frequently associated with diseased vessels and their possible contribution to vascular pathology has been recently suggested in atherosclerosis.[18–24]

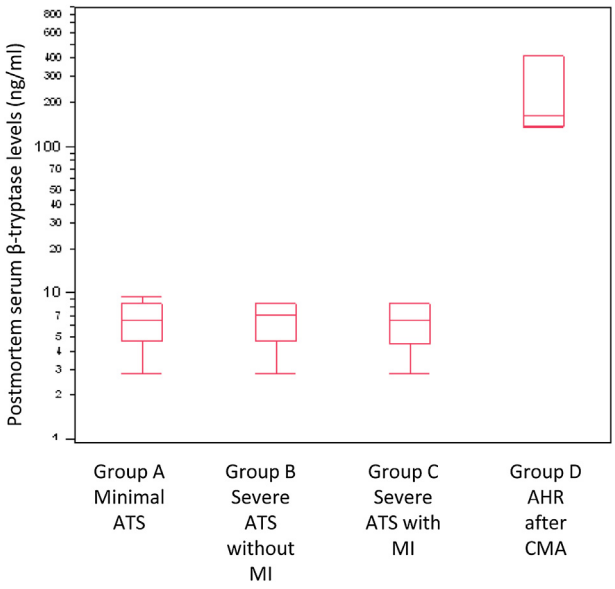

Fig. 3. Distribution of β-tryptase levels in the studied groups. Box plots show the median, interquartile range and outliers of β-tryptase levels. ATS: atherosclerosis, MI: myocardial infarction, AHR: acute hypersensitivity reactions, CMA: contrast material administration.

Activated mast cells release both preformed and newly synthesized mediators. The former include histamine, heparin and several proteases such as tryptase, chymase, mast cell carboxypeptidase and cathepsin G. The latter include lipid mediators such as prostaglandin D(2) and leukotriene C(4) as well as cytokines and interleukins.[25]

In addition to macrophages and other inflammatory cells, mast cells also reside in the arterial adventitia and intima.[26] Locally activated mast cells have been postulated to participate not only in developing but also weakening atherosclerotic plaques.[4,5]

An increase in mast cells in the adventitia overlying ruptured plaques was noted with the suggestion that mast cell-derived histamine might induce coronary spasms thus predisposing the plaque to rupture.[18]

In the intima, mast cells could be involved in the transformation of macrophages into foam cells.[26] Indeed, neutral protease released from mast cells can degrade apolipoprotein B (apoB), thereby facilitating low-density lipoprotein (LDL) accumulation in macrophages and, finally, foam cell formation from macrophages. Moreover, LDL accumulation in smooth-muscle cells mediated by heparin released by mast cells could promote foam cell formation from smooth-muscle cells and inhibit smooth-muscle cell proliferation, consequentially contributing to media loss and arterial wall thinning.[26,27]

In studies using mice lacking histamine, a reduction in plaque area, expression of metalloproteinase and expression of inflammatory cytokine genes was observed. The results of these studies therefore provided strong evidence that mast cell-derived histamine is an important driver of plaque expansion and instability.[28] Zhi and co-workers postulated that tryptase participates directly in atherosclerotic plaque hemorrhage in a group of mice overexpressing tryptase by promoting angiogenesis and regulating the balance between plasminogen activator inhibitor-1 and tissue plasminogen activator.[29]

Significant mast cell accumulation at the site of atheromatous erosion or rupture has been described in clinical studies. Based on these findings, associations between blood tryptase levels and atherosclerotic plaque instability have been suggested by some authors.[4,5,8,30,31]

However, due to divergent clinical observations, antithetic conclusions were drawn by different research groups concerning the diagnostic relevance of tryptase levels in acute coronary syndromes. Indeed, while some authors report positive association between plaque instability and tryptase, other authors found a lack of association and concluded that increased mast cell activity in the atherosclerotic plaque is not reflected by higher tryptase concentrations. Thus, conclusive answers on this topic are presently unavailable.[3,8,31,32]

The results of our investigations indicate that increased postmortem serum β-tryptase levels with normal total IgE values can be occasionally observed in cardiac deaths with coronary atherosclerosis disease of various severities. Mast cells and eosinophils within coronary plaques may be observed in some of these cases. These finding partially concur with those of former studies realized by Perskvist et al.[10,11] and Edston and van Hage-Hamsten[12] on autopsy cases.

Perskvist et al.[10,11] analyzed α + β-tryptase levels and mast cell infiltration in heart sections in anaphylactic deaths, sudden natural deaths and drug overdose fatalities following heroin, amphetamine or methadone injection. These authors found that sudden natural deaths were not systematically characterized by increases in α + β-tryptase levels. Moreover, several cases of heroin injection-related deaths had a degree of cardiac mast cell infiltration similar to that observed in anaphylactic deaths, higher α + β-tryptase concentrations and an absence of specific IgE against morphine. Based on these results, the authors concluded that mast cell degranulation into the interstitial spaces of myocytes may play a role in coronary spasms and cardiac disturbances potentially leading to death following drug injection.

Edston and van Hage-Hamsten[12] evaluated IgE and tryptase in 29 cases of sudden deaths from coronary artery thrombosis and 27 control subjects. The authors did not observe significant differences in postmortem serum tryptase and IgE concentrations between groups, though mast cells in the coronary arteries were higher in cardiac deaths than control cases.

In the realm of forensic pathology, besides cases of hypersensitivity reactions, increased postmortem serum β-tryptase levels along with normal IgE values have been described in numerous situations unrelated to anaphylaxis. These include salicylate overdose, crushing blunt force trauma, drug injection (mostly heroin) related-deaths and sudden infant death syndrome. Though a direct mechanical injury to tissue mast cells with subsequent degranulation and a non-immunologic (non-IgE mediated) mast cell degranulation promoted by opiates like morphine and heroin have been evocated to explain some of these results, conclusive interpretations cannot be given at present.[15,16,33–38]

To conclude, postmortem serum β-tryptase concentrations over the clinical cutoff value of 11.4 ng/ml and even over the reference value of 45 ng/ml proposed to discriminate anaphylactic from nonanaphylactic deaths may be found, though inconstantly, in coronary atherosclerosis disease of various degrees. Pathologic examination of coronary arteries may reveal in these cases eosinophil and mast cell accumulation. These findings corroborate former clinical and forensic observations, suggesting that activated mast cells in an atherosclerotic plaque do not systematically determine β-tryptase increases in systemic circulation.

A second conclusion that might be drawn from the present investigation is that the reference value of 45 ng/ml proposed to discriminate anaphylactic from nonanaphylactic deaths in the forensic setting must be handled with extreme caution. Indeed, even though postmortem serum β-tryptase concentrations in acute hypersensitivity reactions after contrast material administration were much higher than those measured in cardiac deaths in our autopsy series, a value of 65 ng/ml (with normal IgE) was found in a

37-year-old man. The cause of death was not established during autopsy in this case and the conclusion of natural death was reached only after all postmortem investigations. These findings emphasize that increased postmortem serum β-tryptase levels alone are an insufficient criterion with which to identify fatal hypersensitivity reactions. Indeed, the establishment of this diagnosis after death requires careful correlation of circumstantial elements, medical history, autopsy and histology findings as well as toxicology and biochemistry results.[16]

5. Conclusion

Increased postmortem serum β-tryptase levels can be observed in cardiac deaths with coronary atherosclerosis disease of various severities. Nevertheless, local mast cell activation in coronary atherosclerosis disease, if any, does not constantly turn into massive β-tryptase release and therefore cannot be ascertained by measurements of β-tryptase in postmortem serum.

Ethical approval

The authors wish to confirm that all cases selected for this work underwent medico-legal autopsies requested by the public prosecutor. Biochemical investigations were performed as part of medico-legal investigations.

Funding

The authors wish to confirm that this study was not financially supported.

The authors wish to confirm that there is not any involvement of study sponsors in the study design, data collection, data interpretation, data analysis, writing the manuscript and submitting the manuscript for publication.

Conflict of interest

The authors wish to confirm that there are no known conflicts of interest associated with this work (*Measurement of β-tryptase in postmortem serum in cardiac deaths*) and there has been no significant financial support for this work that could have influenced its outcome.

The authors have no controlling interests in the Journal of Forensic and Legal Medicine.

The authors wish to confirm that there is not any actual or potential conflict of interest including any financial, personal or other relationship with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, this work.

The authors confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

The authors further confirm that the order of authors listed in the manuscript has been approved by all of us.

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